

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)
Raymond M. GLADUE, et al.) Group Art Unit: To Be Assigned
Rule 53 (b) Division of) Examiner: To Be Assigned
Application Number:
09/529,021)
Filed: April 6, 2000)
For: DHA-CONTAINING NUTRITIONAL COMPOSITIONS AND METHODS FOR
THEIR PRODUCTION)

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of this divisional application of U.S. Application No. 09/529,021, filed April 6, 2000, please enter the following preliminary amendment.

IN THE CLAIMS:

Kindly cancel claims 1-24.

Please add new claims 25-60.

--25. A particulate material containing phospholipids with docosahexaenoic acid (DHA) residues and arachidonic acid residues prepared by drying a slurry comprising a polar lipid extract from DHA-containing microbes and ARA-containing microbes.

26. The particulate material of claim 25, wherein the mean particle size is between 5 microns and 10 microns.

27. The particulate material of claim 25, wherein the slurry is dried by spray drying.
28. The particulate material of claim 25, wherein at least 10% of the fatty acid residues in lipids of said DHA-containing microbes are DHA residues.
29. The particulate material of claim 25, wherein at least 10% of the fatty acid residues in polar lipids of said DHA-containing microbes are DHA residues.
30. The particulate material of claim 25, wherein said ARA-containing microbes are fungi.
31. The particulate material of claim 25, wherein said ARA-containing microbes are *Mortierella sp.*
32. The particulate material of claim 25, wherein said DHA-containing microbes are dinoflagellates.
33. The particulate material of claim 25, wherein said DHA-containing microbes are *Cryptocodinium cohnii*.
34. A method for preparing a DHA- and ARA-containing particulate material comprising drying a slurry containing polar lipids extracted from dinoflagellates and fungi, wherein the dried material is in the form of particles having a mean particle diameter between 5 and 10 microns.
35. A method for preparing a DHA and ARA-containing particulate material comprising lysing DHA-containing and ARA-containing microbial cells; extracting lysed cells with solvent; separating a polar lipid fraction from the extract; and drying the polar lipid fraction, with or without addition of other nutrients, to form a particulate material.
36. The method of claim 35, wherein the polar lipid fraction is an aqueous slurry which is dried by spray drying.

37. The method of claim 35, wherein the DHA-containing microbial cells are dinoflagellate cells.
38. The method of claim 35, wherein the DHA-containing microbial cells are cells of *Cryptocodinium cohnii*.
39. The method of claim 35, wherein the ARA-containing microbial cells are fungal cells.
40. The method of claim 35, wherein the ARA-containing microbial cells are cells of *Mortierella sp.*.
41. An aqueous emulsion or suspension containing phospholipids with docosahexaenoic acid (DHA) residues and arachidonic acid (ARA) containing residues prepared by homogenizing with water a polar lipid extract from DHA-containing microbes and ARA-containing microbes.
42. The emulsion or suspension of claim 41, wherein at least 10% of the fatty acid residues in lipids of the DHA-containing microbes are DHA residues.
43. The emulsion or suspension of claim 41, wherein at least 10% of the fatty acid residues in polar lipids of said DHA-containing microbes are DHA residues.
44. The emulsion or suspension of claim 41, wherein said DHA-containing microbes are dinoflagellates.
45. The emulsion or suspension of claim 41, wherein said DHA-containing microbes are *Cryptocodinium cohnii*.
46. The emulsion or suspension of claim 41, wherein said ARA-containing microbes are fungi.
47. The emulsion or suspension of claim 41, wherein said ARA-containing microbes are *Mortierella sp.*

48. A composition comprising a particulate material containing phospholipids with DHA and ARA prepared by drying a slurry comprising a polar lipid extracts from DHA-containing microbes and ARA-containing microbes, and a meal containing protein, carbohydrate, or both.

49. The composition of claim 48, wherein meal comprises microbial cells or cell fragments.

50. The composition of claim 48, wherein the microbial cells or cell fragments are from *Chlorella*.

51. The composition of claim 48, wherein the microbial cells or cell fragments are from *Cryptocodinium*.

52. The composition of claim 48, wherein the microbial cells or cell fragments are from a yeast.

53. The composition of claim 48, wherein the microbial cells or cell fragments are from *Mortierella*.

54. A method of aquaculture comprising
feeding particulate material containing a polar lipid extract from microbes comprising phospholipid with DHA residues and phospholipid with ARA residues to live larval feed organisms comprising artemia, rotifers, or a combination thereof to enrich DHA and ARA levels in the larval organisms; and

feeding DHA- and ARA-enriched live larval organisms to fish larva, bivalves, crustaceans, or a combination thereof.

55. A method of aquaculture comprising
feeding particulate material containing a polar lipid extract from microbes comprising phospholipid with DHA residues and phospholipid with ARA residues to bivalves and/or crustaceans.

56. The method of claim 54 or 55, wherein particulate material containing phospholipid with DHA residues and ARA residues has mean particle size from about 5 microns to about 10 microns.

57. The method of claim 54 or 55, wherein particulate material containing phospholipid with DHA residues and ARA residues comprises DHA and EPA in ratio of at least 300:1.

58. The method of claim 54 or 55, wherein particulate material containing phospholipid with DHA residues and ARA residues further comprises vitamins, amino acids, or both.

59. The method of claim 54 or 55, wherein particulate material containing phospholipid with DHA residues and ARA residues further comprises *Chlorella* biomass.

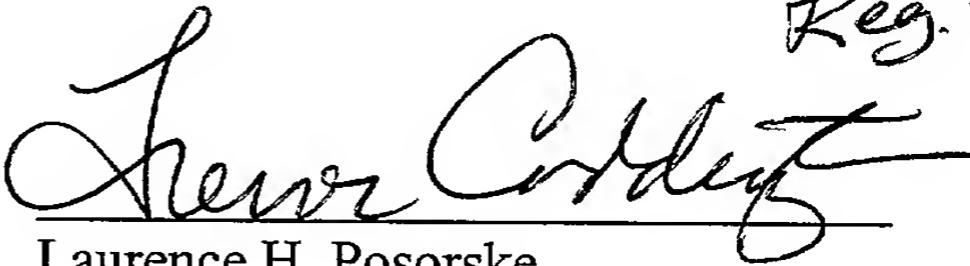
60. The method of claim 54 or 55, wherein particulate material containing phospholipid with DHA residues and ARA residues is prepared by spray-drying a phospholipid-containing byproduct produced in refining a lipid extract from microalgae. --

REMARKS

Applicant believes that no new matter is introduced in the filing of this Preliminary Amendment. Applicant respectfully requests examination of the above-named application in view of the present amendments.

Respectfully submitted,

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